

Presentation of unpaired unconditional stimuli during extinction reduces renewal of conditional fear and slows re-acquisition

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USs during extinction reduce fear renewal

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Abstract

Past research has shown that presenting unconditional stimuli (US) during extinction training, either paired with the conditional stimulus (CS) or explicitly unpaired, can reduce spontaneous recovery and slow the re-acquisition of conditional fear. Whether contextual renewal of conditioned fear as indexed by electrodermal responses and self-report measures of anxiety and CS evaluations is also reduced after presentation of paired or unpaired USs during extinction training is currently unclear. Three groups of participants (Paired, Unpaired, Standard Extinction) completed a sequence of habituation, acquisition, extinction, renewal, and re-acquisition phases. During extinction, five CS-US pairings were presented in group Paired, whereas five USs were presented alone in group Unpaired. No USs were presented during standard extinction. For all groups, extinction was conducted in a context that was different from that of the other phases. Extinction of differential electrodermal responding was evident in groups Unpaired and Standard, but not in group Paired. Contextual renewal and fast re-acquisition, as indexed by differential electrodermal responding, were evident in group Standard, but not in group Unpaired and differential electrodermal responding persisted in group Paired. After extinction, self-reported anxiety was higher in groups Paired and Unpaired, but differential CS evaluations were evident in group Paired only. The current results suggest that presenting additional unpaired USs during extinction training strengthens extinction and protects against the renewal of differential electrodermal responding.

Key words: Fear conditioning, partially reinforced extinction, unpaired extinction, Renewal, Re-acquisition, Return of fear, electrodermal responses.

Relapse of fear after successful treatment with behaviour based psychological interventions remains a major problem for clinical psychological science (Bouton 2000). While dissatisfactory from an applied clinical perspective, this phenomenon is not surprising given our current understanding of how fear is acquired, maintained, and reduced. There is now a consensus that Pavlovian learning due to the pairing of a conditional stimulus (CS) with a fear evoking, unconditional stimulus (US) is crucial in the acquisition of fear (for a review see Mineka & Zinbarg, 2006) and Pavlovian extinction is thought to be a core process in the reduction of fear during behaviour based interventions, most clearly exemplified in exposure based approaches (Craske et al., 2014; Craske et al., 2018). Extinction is achieved by presenting the CS repeatedly alone, without the US, until responding to the CS declines and is comparable to that elicited by a stimulus never paired with the US. This reduction in responding does not, however, indicate that the association between CS and US that was acquired during acquisition has been eliminated (for reviews see Bouton, 2000; Craske et al., 2018). Rather, it indicates that a second inhibitory association has been formed between the CS and US. Whether the CS will elicit a conditional response after successful extinction. i.e., whether relapse is observed, depends on which association is dominant at test. Relapse is more likely with a longer time interval since extinction (spontaneous recovery), outside the environment of extinction (renewal), or after another traumatic experience (reinstatement).

One approach to reducing relapse is to strengthen the inhibitory association acquired during extinction training (see Lipp et al., 2020). Although counterintuitive on first encounter, research in rodents has provided some evidence that the occasional presentation of the US during extinction, either paired with the CS or explicitly unpaired, can prevent the return of fear. Bouton et al. (2004) demonstrated that paired and unpaired presentations of the US strengthened extinction learning as indicated by slower re-acquisition and Gershman et al. (2013) showed that occasional CS-US pairings presented at a gradually decreasing density reduced spontaneous recovery and reinstatement. Bouton et

al. (2004) offered a trial signalling account to explain their findings suggesting that animals learn not only the relationship between the stimuli presented on a given trial, but also predict the nature of subsequent trials presented in a particular phase. During acquisition, animals learn that CS-US pairings will be followed by CS-US pairings and during standard extinction, animals learn that CS alone trials will follow CS alone trials. Presenting occasional CS-US pairings during extinction enables the learning that CS alone trials can be followed by CS-US trials and conversely, CS-US trials by CS alone trials. The latter learning weakens the expectation that CS-US trials follow CS-US trials acquired during acquisition and, thus, slows re-acquisition. Gershman et al. (2013) explained their findings within a latent state account that postulates that animals will segment acquisition and extinction phases into distinct memories labelled by different latent states. Scheduling CS-US pairings in a gradually reducing density attenuates the transition between acquisition and extinction phases and prevents the formation of separate latent states which, in turn, allows the CS alone trials to weaken the association learned during acquisition.

The effect of presenting occasional unpaired USs during extinction has received less discussion in past literature. Vervliet et al. (2010) offered several potential explanations for their effect. Presenting the US alone may result in habituation to the US or US devaluation (Holland & Rescorla, 1974) which reduces conditional responding to the CS in a post-extinction test. This interpretation receives support from studies that assess the effect of US alone exposures relative to CS alone exposures during extinction (Haesen & Vervliet, 2015; Leer et al., 2018), but is inconsistent with results from animal research (Thomas et al., 2005) and findings that US alone presentations during extinction do not reduce perceived US aversiveness (Leer et al., 2018). Second, presenting the US alone during extinction may result in inhibitory conditioning to the CS when presented alone in the presence of a slightly excitatory context, but this explanation is inconsistent with findings from animal research as well (Thomas et al., 2005). Finally, and similar to Gershman et al.'s (2013) latent state account, presenting the US during

extinction training may reduce the distinction between acquisition and extinction learning episodes and thereby allow the CS-US association to be weakened. Thomas et al. (2005) proposed a hybrid theory to account for their findings, combining Bouton's (2002) notion of an inhibitory association being formed between CS and US during extinction that competes with the CS-US association acquired during acquisition with Rescorla-Wagner's (1972) proposal that extinction weakens the CS-US association formed during acquisition. This weakening of the CS-US association is said to be stronger if responding is evoked in the presence of the CS during extinction (for instance by unpaired USs). At extinction test, the new inhibitory association suffers a larger generalization decrement than does the residual CS-US association, which may lead to the return of fear. Thus, presenting unpaired USs during extinction training does not strengthen the inhibitory association formed between CS and US, but weakens the CS-US association formed during acquisition.

In humans, only a few studies have assessed the effects of presenting occasional CS-US pairings or unpaired USs during extinction training (Culver et al., 2018; Shiban et al., 2015; Thompson et al., 2018; van den Akker et al., 2015; Vervliet et al., 2010). Shiban et al. aimed to extend the work by Gershman et al. (2013) comparing the effects of gradual extinction training with standard extinction on reinstatement assessed 24 hours after extinction. The groups did not differ in electrodermal responses or ratings of US expectancy, but differences emerged in fear potentiated startle, such that at the reinstatement test, the difference between startle blinks during CS+ and CS- was larger after standard extinction than after gradual extinction. This result should be interpreted with caution, however, as the sample assessed comprised 23 participants only, 11 in group Gradual, and this difference between groups was present at the end of extinction as well.

Culver et al. (2018) examined spontaneous recovery, re-acquisition and re-extinction of electrodermal responses, US expectancy, and CS pleasantness ratings after extinction with additional CS-US pairings or standard extinction. There was some evidence for slower re-acquisition of

differential electrodermal responses after paired extinction training and spontaneous recovery of US expectancy was reduced. CS pleasantness ratings were not affected by partially reinforced extinction. Vervliet et al. (2010) assessed whether unpaired US presentations during extinction reduced ABA renewal of differential electrodermal responding and US expectancy. Renewal of differential electrodermal responding and differential US expectancy were numerically reduced after extinction training with explicitly unpaired USs, although the latter finding needs to be interpreted with caution as it was not statistically significant, presumably due to low power.

Two studies have compared the effects of paired and unpaired USs on extinction learning and relapse within the same study. Van den Akker et al. (2015) used an appetitive human conditioning paradigm assessing participants' expectation and desire for an appetitive US (chocolate mousse) and their evaluation of the CSs. In the paired extinction procedure, two USs were presented early during extinction training (trials 2 and 6 of 40 extinction trials), whereas USs were presented unpaired during the corresponding intertrial intervals in the unpaired extinction condition. Re-acquisition of US expectancies was slower in the paired and unpaired extinction groups relative to standard extinction. Desire for chocolate mousse and CS pleasantness did not differ between groups. Thompson et al. (2018) assessed the effects of extinction with no USs, paired USs, and unpaired USs on spontaneous recovery, reinstatement, and re-acquisition measuring electrodermal responses during, and CS pleasantness ratings after each of the experimental phases. Spontaneous recovery, assessed after a break of 10 min following extinction, was greater after standard extinction than in the paired and the unpaired extinction groups. Groups did not differ in the extent of electrodermal reinstatement, but this result is difficult to interpret, as differential responding during reinstatement test was not evident in any group. Notably, re-acquisition of differential electrodermal responses was reduced in the group that had received unpaired USs during extinction training relative to both standard and paired extinction. Differential ratings of CS pleasantness were evident after paired extinction training, but not after

standard or unpaired extinction likely due to the continued pairing of CS+ and US. This across group difference was maintained after tests for spontaneous recovery and reinstatement. After re-acquisition, all groups demonstrated differential CS evaluations that did not differ across groups.

Taken together, past research suggests that paired and unpaired presentations of the US during extinction training may reduce spontaneous recovery of conditional fear as indexed by electrodermal responses (Culver et al., 2018; Thompson et al., 2018) and slow the re-acquisition of conditional responding (Culver et al., 2018; Thompson et al., 2018; van den Akker et al., 2015). Moreover, unpaired presentations of the US during extinction training may reduce renewal of electrodermal responding (Vervliet et al., 2010). The current study was designed to add to this literature and to extend on the findings reported by Vervliet et al. (2010) and Thompson et al. (2018), by assessing whether the presentation of additional CS-US pairings, or of unpaired USs during extinction, would reduce ABA renewal and slow subsequent re-acquisition. Specifically, and extrapolating from past empirical findings, we hypothesized that after standard extinction, renewal of differential electrodermal responding would be evident and re-acquisition readily observed of differential electrodermal responding. We predicted that presenting additional USs during extinction training would reduce electrodermal renewal and re-acquisition regardless of whether they were presented paired or unpaired. Based on past literature, these predictions are made strongly for conditional fear as indexed by electrodermal responses. Based on past research (Thompson et al., 2018; Waters et al., 2018) we expected no between group differences in self-report measures of subjective anxiety and of CS pleasantness and arousal.

Method

Participants

Sample size was based on a power analysis using G*Power (Faul et al., 2007) which, assuming a small to moderate effect size ($f=.18$) in a mixed design and a correlation of .5 between repeated measures,

recommended a total sample size of 78 participants to achieve a power of .80 to detect a significant effect at $\alpha = .05$. To permit full counterbalancing of the factors ‘nature of the CS+’, ‘nature of the first CS in each phase’, and stimulus set that served as CS’ group size was set to be 32. One hundred and three first year university psychology students (77% Female; 23% Male) between the ages of 17 and 39 years of age ($M = 20.36$, $SD = 4.23$) volunteered participation in exchange for course credit and provided informed consent. Three participants discontinued the experiment due to high self-reported fear and skin conductance data from one participant were not registered due to a technical error. Due to an assignment error, three participants allocated to Group Unpaired were presented with the control treatment. Participants who volunteered beyond the prespecified group size of 32 and who provided valid data were retained. Thus, the final sample comprised 100 participants with usable self-report data (Paired = 35; Unpaired = 29; Standard = 36) and 99 participants with usable SCR data (Paired = 34; Unpaired = 29; Standard = 36).

Material and Measures

Questionnaires for anxiety and depression symptoms. The State-Trait Anxiety Inventory for Adults (STAI; Spielberger et al., 1983) was used to assess anxiety symptoms. The STAI comprises two 20 item scales designed to differentiate between state and trait anxiety. Cronbach’s alpha in the present study was .94 for State Anxiety and .93 for Trait Anxiety. The Penn State Worry Questionnaire (PSWQ; Meyer et al., 1990) was used to measure trait worry. Cronbach’s alpha in the present study was .90. The Mood and Anxiety Symptom Questionnaire (MASQ; Watson et al., 1995) has two subscales assessing anxious arousal and anhedonic depression. Cronbach’s alpha was .87 for the anxious arousal scale and .94 for the anhedonic depression scale. The Mood and Feelings Questionnaire (MFQ; Angold et al., 1995) assesses core depressive symptomology. Cronbach’s alpha in the present study was .87. The short version of the Intolerance of Uncertainty Scale (IUS-12; Carleton et al., 2007) measures beliefs about and reactions to uncertainty, ambiguous situations, and the

future. Cronbach's alpha was .89.

Conditional and unconditional stimuli. The CSs were pictures of non-fear relevant animals, two fish and two birds as used by Thompson et al. (2018). Each participant saw one set of bird and fish and sets were counterbalanced across participants. The nature of the CS+, fish or bird, was counterbalanced across participants. CS+ and CS- were displayed for 6s during all phases except during acquisition when CSs+ were displayed for 8s. During acquisition, a 3s sound US (a metal fork scraping on slate; 100dBA; see Neumann & Waters, 2006; Neumann et al., 2008) was presented during the last three seconds of the presentation of the 8s CS+. On the occasional CS+-US trials presented during extinction in group Paired, US onset coincided with the offset of the 6s CS+. During re-acquisition, the US was presented 3s after the onset of the 6s CS. The US was presented on 100% of CS+ trials during acquisition and on 50% of CS+ trials during re-acquisition for all participants and during the extinction phase in groups Paired and Unpaired (see Procedure). The intertrial intervals varied randomly between 25-30s in all phases. During intertrial intervals the screen remained the same colour as the assigned context and a black fixation cross was displayed in the centre of the screen to maintain participants' attention.

Context. The screen background colour provided the context stimulus and was manipulated in an ABA sequence with a random allocation of two of three colours (blue, yellow, and pink). One colour was used as the background during habituation, acquisition, renewal, and re-acquisition (context A), whereas the second (context B) was used during extinction.

Physiological recordings. Skin conductance was recorded using two self-adhesive electrodes pre-gelled with isotonic electrolyte (Biopac systems EL507), attached to the thenar and hypothenar eminences of the non-dominant hand, and acquired using a Biopac data acquisition system (Model MP150) with a sampling frequency of 2000Hz via an EDA100C amplifier. Respiration was recorded using a Biopac TSD201 transducer connected to an RSP100C respiration amplifier, to monitor for

respiratory influences on SCRs. Responses were scored using AcqKnowledge software Version 5.0.

Subjective anxiety. Participants rated their subjective anxiety using a unipolar Likert scale ranging from 0 (not at all anxious) to 10 (very anxious). Ratings were provided before and after habituation, and after acquisition, extinction, renewal, and re-acquisition.

US intensity and pleasantness ratings. Participants rated the sound US on intensity (0 - not at all intense to 10 - very intense) and pleasantness (0 - not at all pleasant to 10 - very pleasant) Likert scales after acquisition and after extinction.

CS pleasantness and arousal ratings. Participants rated the CS+ and CS- before and after habituation, and after acquisition, extinction, renewal, and re-acquisition using a dial and button device (ShuttleXpress). Participants moved the cursor along a SAM-scale (self-assessment mannequin; Bradley & Lang, 1994) to provide subjective ratings of CS pleasantness and arousal. Pleasantness was rated using a two tailed Likert scale ranging from 1 (very unpleasant) to 5 (neutral) to 9 (very pleasant) and arousal was rated using a two tailed Likert scale ranging from 1 (very calm) to 5 (neutral) to 9 (very aroused). Rating scales were presented on the same background colour as used for the training phase that preceded them, so ratings before and after habituation, after acquisition, renewal, and re-acquisition were done in context A, whereas ratings after extinction were done in context B.

Contingency awareness. After the acquisition phase, participants were verbally asked “Did you notice if one animal was paired with the tone?” The responses were recorded verbatim. Participants were considered to have contingency awareness if they identified the correct CS+ animal.

Procedure

Ethics approval was obtained through Griffith University Human Research Ethics Committee (GU Ref No: 2019/165). Participants were recruited through the university research participation scheme and received course credit. On arrival participants washed their hands and were seated in a participant cubical within a control room that was monitored by a closed-circuit camera. Participants

provided informed consent and were informed that they would see pictures of animals on the screen and hear a loud sound through the headphones. They were told that at times, questions would appear on the screen asking them to rate the pleasantness and arousal of each animal using the SAM scale. Participants were shown how to provide their ratings and asked to report their subjective anxiety. A respiration belt was attached around their chest to identify any respiration induced artefacts and movements that might affect skin conductance. Two electrodes were attached to the thenar and hypothenar eminences of the participant's non-dominant hand to record skin conductance. Participants were informed that their movements were monitored via the internal camera but that recordings were not kept. Headphones were then attached and the researcher left the room.

Habituation phase. After providing online SAM ratings of CS pleasantness and arousal, participants were presented with a random sequence of 4 CS+ and 4 CS- trials in context A, with no US presentation. After the habituation sequence, the SAM ratings were again provided. Participants were asked to take a break and take off their headphones and the researcher entered the participant's room to record the subjective anxiety.

Acquisition phase. Participants were presented with a random sequence of 8 CS+ and 8 CS- trials in context A. The first two trials were a CS+ and a CS- (order counterbalanced across participants) and no more than two consecutive trials of the same CS were presented throughout the phase. The US was presented during the last 3 seconds of each CS+ trial. After the end of acquisition, participants rated CS pleasantness and arousal online, and the researcher entered the participant's room to record the participant's subjective anxiety rating, contingency awareness, US intensity, and US pleasantness ratings. Headphones were replaced and the researcher left the room.

Extinction phase. Participants were presented with 24 CS+ and 24 CS- trials in context B in all conditions. In the Paired condition, the US was presented during 5 CS+ trials. In the Unpaired condition, the US was presented at the mid-point of the intertrial interval either before or after a CS+

presentation on 5 trials. No USs were presented in the Standard extinction condition. To schedule the additional USs, the extinction sequence was divided into tertiles, each comprising 8 CS+ and 8 CS- trials, with the US presented randomly at the offset of two CS+ trials (paired) or during the intertrial interval after two CS+ trials (unpaired) in the first and second tertiles and at the offset of one CS+ trial in the last tertile (see Thompson et al., 2018). The US was not presented during (Paired) or between (Unpaired) consecutive CS+ trials in each block. After the end of extinction, participants again rated CS pleasantness and arousal online. The researcher entered the participant's room to record the subjective anxiety rating and ratings of US intensity and US pleasantness. Participants were then given a 10 min break during which the electrode leads were removed and they could stretch their legs. They were given a word search puzzle to complete as a filler task.

Insert Figure 1 about here

Renewal. After the break, the electrode leads were reattached, and headphones placed. The renewal phase, presented in context A, consisted of 4 presentations of each CS with no US. After completion of the phase, CS pleasantness and arousal ratings were recorded online and participants provided their subjective anxiety rating as before.

Re-acquisition phase. Participants were presented with 8 CS+ and 8 CS- trials in context A. The US was presented during the first CS+ trial and on three randomly selected CS+ trials (50% reinforcement). After completion of the phase, the ratings of CS pleasantness and arousal were completed online. Participants provided their final subjective anxiety rating and the SCR electrodes and the respiration belt were removed. Participants then completed the questionnaires, were debriefed, thanked, and awarded course credit.

Response Definitions and Data analysis

Skin Conductance Responses. The magnitude of the skin conductance responses (SCRs) elicited by each CS was scored within two latency windows: first interval responses (FIR) and last

interval responses (LIR; Prokasy & Kumpfer, 1973). Each SCR was scored as the difference between the trough and apex of the curve and expressed in microSiemens (μ S). First interval responses (FIR) commenced within 1-5 s after stimulus onset and late interval responses (LIR) commenced within 6-10 s after CS onset to reflect the response to the 3 s US on reinforced CS+ trials (which started 5s after CS+ onset during acquisition and 6s after CS+ onset during extinction in group Paired) or to US absence on trials without the US (Prokasy & Kumpfer, 1973). The only exception to this was in the re-acquisition phase when the US occurred 3s after CS+ onset. Therefore, the latency windows were changed for FIR to 0-3s and for LIR to 4 – 8 s. Skin conductance responses were square root transformed in order to normalise their distribution (Venables & Christie, 1980).

Analyses were conducted separately for FIRs and LIRs for the habituation (FIR only), acquisition, extinction, renewal, and re-acquisition phases using 3 (Group: Paired; Unpaired; Standard) \times 2 (CS: CS+, CS-) \times n (Block: average across two trials) linear mixed models for repeated measurements with Satterthwaite's approximation for degrees of freedom. In post hoc comparisons that assessed the extent of differential responding to CS+ and CS-, differences in differential responding were concluded if the 95% confidence intervals of the difference scores between CS+ and CS- did not overlap. Follow-up comparisons for main effects or interactions were Bonferroni corrected to control for the accumulation of error due to multiple comparisons, i.e., assessed against a criterion of .05/(number of tests conducted).

Subjective ratings. Participants' CS evaluations and anxiety ratings were analysed using 3 (Group: Paired; Unpaired; Standard) \times 6 (Phase: Pre-habituation, Post-habituation, Post-acquisition, Post-extinction; Post-renewal; Post-Re-acquisition) \times 2 (CS: CS+, CS-) and 3 (Group: Paired; Unpaired; Standard) \times 6 (Phase: Pre-habituation, Post-habituation, Post-acquisition, Post-extinction; Post-renewal; Post-Re-acquisition) mixed model factorial ANOVAs, respectively. The multivariate solution of the ANOVA (Pillai's Trace) is reported and the alpha criterion was set to .05. As for

electrodermal data, follow-up comparisons were Bonferroni corrected.

Results

Preliminary analyses

The three participant groups did not differ in age, gender composition or the number of participants who could report the experimental contingencies or on any of the self-report measures (see Table 1). Participants evaluated the sound US as more pleasant ($M = 0.60$, $SD = 1.25$ vs. $M = 0.38$, $SD = 0.84$, $F(1,97) = 4.80$, $p = .031$, $\eta_p^2 = .047$) and less intense ($M = 7.65$, $SD = 2.04$ vs. $M = 8.06$, $SD = 1.46$, $F(1,97) = 5.08$, $p = .027$, $\eta_p^2 = .050$) after extinction than after acquisition, but there was no difference between groups nor an interaction involving the group factor, all $F(2,97) < 2.63$, $p > .076$, $\eta_p^2 < .052$.

Insert Table 1 about here

Electrodermal responses: FIR

Habituation and acquisition. First interval responses elicited by CS+ and CS- in the three groups across the phases of the experiment are displayed in Figure 2. Electrodermal responses during habituation did not differ as a function of any of the experimental factors, all $F < 2.05$, $p > .152$. During acquisition, electrodermal FIRs were larger to CS+ than CS-, main effect of CS, $F(1,917.50) = 278.44$, $p < .001$, however, this difference was qualified by Group \times CS, $F(2, 917.65) = 5.39$, $p = .005$, and CS \times Block interactions, $F(6,838.87) = 32.99$, $p < .001$. The Group \times CS interaction indicates that, although responses to CS+ were larger than responses to CS- in all three groups, all $F_s > 42.90$, $p < .001$, the extent of differential responding in group Paired was larger than in group Unpaired, but not in group Standard. The CS \times Block interaction reflects larger responses to CS+ than to CS- on blocks 2-4, all $F_s > 87.30$, $p < .001$, but not on block 1, $F(1, 968.93) = 0.37$, $p = .545$.

Extinction. As shown in Figure 2, differential responding during extinction differed across groups, with group Paired retaining differential responses to CS+ than CS- throughout. This impression

was confirmed by the analysis which yielded main effects for Group, $F(2,773.75) = 24.11, p < .001$, and CS, $F(1,2463.44) = 212.36, p < .001$, as well as Group \times CS, $F(2,2463.40) = 108.69, p < .001$, and Group \times CS \times Block interactions, $F(22,2881.82) = 2.02, p = .003$. The three-way interaction was driven by larger responses to CS+ than CS- in group Paired on blocks 2-12 (all $p < .001$) and in group Standard on block 6 ($p < .001$), whereas there was no significant difference in group Unpaired, all $F < 6.60, p > .009$ (Bonferroni corrected $p_{crit} = .001$).

Insert Figure 2 about here

Renewal. Electrodermal FIRs were larger to CS+ than to CS- in groups Standard and Paired, but not in group Unpaired. The analysis confirmed this impression yielding main effects for CS, $F(1,441.87) = 41.68, p < .001$, and Block, $F(1,533.91) = 6.87, p = .009$, as well as a Group \times CS interaction, $F(2,441.34) = 9.33, p < .001$. The Group \times CS interaction was driven by larger responses to CS+ than CS- in groups Standard, $F(1,434.871) = 15.08, p < .001$, and Paired, $F(1,435.10) = 50.53, p < .001$, but not in group Unpaired, $F(1,452.89) = 0.34, p = .561$.

Re-acquisition. Larger responses to CS+ than to CS- were apparent in all groups, but the extent of differential responding and the speed at which it was acquired differed across groups. The omnibus analysis confirmed this impression yielding a main effect for CS, $F(1,822.19) = 128.71, p < .001$, and a Group \times CS interaction, $F(2,822.05) = 15.91, p < .001$. The interaction reflects that although responses to CS+ were larger than to CS- in groups Standard, $F(1,825.06) = 34.62, p < .001$, Paired, $F(1,814.67) = 126.84, p < .001$, and Unpaired, $F(1,826.14) = 8.29, p = .004$, the extent of differential responding was larger in group Paired than in groups Unpaired and Standard. An exploratory analysis including only Block 1 of re-acquisition confirmed larger responses to CS+ than to CS- in groups Standard, $F(1,196.83) = 10.89, p = .001$, and Paired, $F(1,200.67) = 24.67, p < .001$, but not in group Unpaired, $F(1,193.37) = 0.04, p = .843$.

Electrodermal responses: LIR

Acquisition. Last interval responses elicited by the US or its absence are presented in Figure 3.

The statistical analyses for acquisition yielded main effects for CS, $F(1,623.15) = 1508.13, p < .001$, and Block, $F(3,733.51) = 19.53, p < .001$, and a CS \times Block interaction, $F(3,790.59) = 23.11, p < .001$. The interaction reflects that responses to the US declined across blocks (block 1 > blocks 2-4, block 2 > block 4, all $p < .001$), whereas responses during CS- remained unchanged.

Insert Figure 3 about here

Extinction. During extinction, electrodermal LIRs were larger during CS+ than during CS- in group Paired whereas there was no difference in the other groups. The analysis yielded main effects for Group, $F(2,1238.73) = 70.53, p < .001$, and CS, $F(1,1881.89) = 194.01, p < .001$, and Group \times CS, $F(2,1883.0) = 131.57, p < .001$, and Group \times CS \times Block interactions, $F(22,2443.66) = 1.69, p = .023$. LIRs during CS+ were larger than responses during CS- in group Paired, $F(1,1875.37) = 466.41, p < .001$, but not in the remaining groups, Standard, $F(1,1933.84) = 3.62, p = .057$, Unpaired, $F(1,844.82) = 1.17, p = .279$.

Renewal. Analysis of LIRs during renewal yielded main effects for Group, $F(2,199.81) = 3.37, p = .037$, and CS, $F(1,304.64) = 5.08, p = .025$. Responses were larger to CS+ than to CS-, but the between group difference (Paired > Standard) was not significant after correction for multiple testing. An exploratory analysis on Block 1 of renewal yielded a Group \times CS interaction, $F(2,168.31) = 3.34, p = .038$, reflecting that differential responding was evident in group Paired, $F(1,173.92) = 6.46, p = .012$, but not in groups Standard, $F(1,165.66) = 0.77, p = .383$, and Unpaired, $F(1,165.59) = 1.34, p = .249$.

Re-acquisition. During re-acquisition, electrodermal LIRs were larger during CS+ than during CS-, $F(1,617.57) = 478.13, p < .001$, a difference that was not affected by any other factor, all $F < 1.65, p > .137$.

Subjective ratings

Subjective anxiety. Figure 4 summarizes the subjective anxiety ratings provided by the participants. Self-reported anxiety increased after acquisition in all groups and returned to baseline levels after extinction in group Standard and after renewal in groups Paired and Unpaired. Self-reported anxiety increased after re-acquisition in all groups. The analysis yielded a main effect of Phase, $F(5,93) = 47.60, p < .001, \eta_p^2 = .719$, and a Group \times Phase interaction, $F(10,188) = 3.93, p < .001, \eta_p^2 = .173$. In all groups, self-reported anxiety did not change from pre- to post-habituation (all $p > .90$), but was higher after acquisition than after habituation, all $p < .001$. In group Standard, self-reported anxiety declined significantly from post-acquisition to post-extinction ($p < .001$), did not change significantly from post-extinction to post-renewal ($p > .95$) and increased again post-reacquisition compared to post-renewal, $p < .001$. In groups Paired and Unpaired only, self-reported anxiety did not decline significantly from post-acquisition to post-extinction (both $p > .235$), but was smaller at post-renewal compared to post-extinction (both $p < .001$), and unlike group Standard, was not elevated after re-acquisition compared to post renewal (both $p > .049$; Bonferroni corrected $p_{crit} = .003$). Self-reported anxiety was significantly lower in group Standard than in group Paired after extinction, $p = .001$, but did not differ between groups at any other measurement point (all $p > .010$; Bonferroni corrected $p_{crit} = .003$).

Insert Figure 4 about here

CS pleasantness ratings. Figure 5 summarizes the CS pleasantness ratings provided between phases. The omnibus analysis yielded main effects for Phase, $F(5,93) = 15.0, p < .001, \eta_p^2 = .446$, and CS, $F(1,97) = 52.05, p < .001, \eta_p^2 = .349$, as well as Phase \times CS, $F(5,93) = 33.62, p < .001, \eta_p^2 = .644$, and Group \times Phase \times CS interactions, $F(10,188) = 3.93, p < .001, \eta_p^2 = .175$. The three-way interaction was followed up by calculating difference scores in evaluation of CS+ and CS-. These differential evaluations of CS+ and CS- were larger in group Paired ($M = 3.40; SD = 3.30$) than in groups Standard ($M = 0.86; SD = 1.87; t(69) = 3.97, p < .001$) and Unpaired ($M = 0.41; SD = 2.57; t(62) = 3.97, p <$

.001) post-extinction. The apparent difference in differential evaluations after renewal was not significant after adjustment for multiple testing, largest t : $t(57.46) = 2.05$, $p = .045$, Bonferroni corrected $p_{crit} = .003$. Participants in all groups rated CS+ as more unpleasant than CS- after acquisition and after re-acquisition, all $p < .001$, and participants in group Paired rated CS+ as more unpleasant than CS- after extinction and after renewal, all $p < .001$.

Insert Figure 5 about here

CS arousal ratings. The CS arousal ratings provided between phases are depicted in Figure 6. The omnibus analysis yielded main effects for Phase, $F(5,93) = 41.62$, $p < .001$, $\eta_p^2 = .691$, and CS, $F(1,97) = 122.61$, $p < .001$, $\eta_p^2 = .558$, as well as Group \times Phase, $F(10,188) = 2.32$, $p = .014$, $\eta_p^2 = .110$, Phase \times CS, $F(5,93) = 46.53$, $p < .001$, $\eta_p^2 = .714$, and Group \times Phase \times CS interactions, $F(10,188) = 3.81$, $p < .001$, $\eta_p^2 = .169$. The three-way interaction was due to larger differential arousal ratings of CS+ and CS- post-extinction in group Paired ($M = 3.60$; $SD = 2.39$) than in groups Standard ($M = 1.11$; $SD = 1.98$; $t(69) = 4.78$, $p < .001$) and Unpaired ($M = 1.0$; $SD = 2.51$; $t(62) = 4.24$, $p < .001$). After renewal, differential CS arousal ratings were larger in group Paired ($M = 2.06$; $SD = 2.27$) than in group Unpaired ($M = 0.52$; $SD = 1.62$; $t(62) = 3.06$, $p = .003$), but not in group Standard after adjustment for multiple testing ($M = 1.0$; $SD = 1.88$; $t(69) = 2.14$, $p = .036$; Bonferroni corrected $p_{crit} = .003$). Participants in all groups rated CS+ as more arousing than CS- after acquisition and after re-acquisition, all $p < .001$. This differential evaluation was also evident in group Paired after extinction and renewal, both $p < .001$, a difference that was not significant in the other groups after adjustment for multiple testing.

Insert Figure 6 about here

Discussion

The present study was designed to assess whether presenting paired or unpaired USs during extinction training would reduce ABA renewal and re-acquisition of differential electrodermal

responding. Extinction training reduced differential electrodermal responding that had been acquired during acquisition in groups Standard and Unpaired, but not in group Paired. The unexpected failure to observe extinction in group Paired prevents a meaningful interpretation of the results observed in this group during renewal and re-acquisition as the differential responding observed in these phases is likely to reflect the continuation of the differential responding seen during the extinction phase. Renewal of differential responding which persisted throughout the renewal phase was observed after standard extinction training, whereas context change did not seem to renew differential electrodermal responding after presentation of unpaired USs during extinction training. Moreover, group Unpaired displayed slower and less pronounced re-acquisition of differential electrodermal responses than was observed after standard extinction.

Self-reported anxiety, which after acquisition was elevated in all three groups, decreased after extinction in group Standard, but not until after renewal in groups Paired and Unpaired and did not increase significantly in these latter groups after re-acquisition. In terms of rated CS pleasantness and arousal, extinction training eliminated the differential evaluations in groups Unpaired and Standard, but not in group Paired and differential arousal ratings were still evident in group Paired after renewal. Thus, it seems that self-reported anxiety tracked the presentation of USs, either paired or unpaired, in the phase that preceded its assessment, whereas ratings of CS pleasantness and arousal tracked the presence of CS-US pairings in the phase that preceded their assessment, regardless of the context in which these pairings were presented.

The finding that presenting five occasional CS-US pairings during extinction training maintained differential electrodermal responding was unexpected, as Thompson et al. (2018), using a similar design, had found uniform extinction across all three groups. However, Thompson et al. controlled the scheduling of the CS-US pairings and did not present an occasional CS-US trial in the last half of the third tertile of extinction training. Rather, USs were presented after the 4th, 8th, 11th, 15th,

and 20th CS+ presented during extinction training. Thus, each CS+-US trial presented during extinction was preceded and followed by a set of CS+ alone presentations. It may be that participants learned this pattern across the repetitions which may have supported lower responding on the CS+ trials immediately after an occasional CS+-US pairing. Moreover, Thompson et al. assessed extinction by comparing responses to the first four CS+ and CS- trials of extinction training with responses to the last four CS+ and CS- trials of extinction training, neither of which contained a US. The schedule of the occasional CS-US pairings was less restricted in the current study with their occurrence randomized within each tertile of extinction. The more irregular schedule throughout the entire extinction phase which permitted the occurrence of USs on the first extinction trial as well as during the last extinction trials may have maintained conditional responding. Given that extinction of differential responding in the presence of occasional CS-US pairings appears to be affected by the presentation schedule, future studies assessing the effects of presenting occasional CS-US pairings during extinction should employ a gradual schedule as suggested by Gershman et al. (2013).

It should be noted, however, that the maintenance of strong differential responding across 48 trials with a reinforcement ratio of 21% is an interesting observation. It suggests that after a period of continuous reinforcement even a very sparse schedule of reinforcement can maintain conditional fear responses. Clinically, this finding may have implications for the maintenance of chronic stress when confronted with intermittent abuse and victimisation¹.

By contrast, it appears that extinction of differential responding is less sensitive to the schedule of occasional USs when presented unpaired with the CSs during extinction training. In the current study, five unpaired USs were presented across the 48 extinction trials (24CS+/24CS-), and the results replicate prior findings that six unpaired USs among 16 extinction trials (8CS+/8CS-) can reduce the ABA renewal of conditional electrodermal responses (Vervliet et al., 2010). Moreover, the present

¹ We would like to thank the anonymous reviewer who pointed this potential application out.

study extends this result to a second test of return of fear, re-acquisition. Renewal of differential electrodermal responding after a context change was clearly evident after standard extinction, but absent in group Unpaired. Neither Vervliet et al. (2010) nor the current data provide any evidence for differential responding in renewal conducted in the original acquisition context in group Unpaired – not even on the first trial of renewal. This may suggest that the addition of unpaired USs did not result in a novel, second learning which has been shown to be context specific, but instead targeted the learning participants had engaged in during acquisition. This interpretation is consistent with the one offered by Vervliet et al. (2010) who, following Rescorla (2001), suggested that the lack of renewal after extinction with occasional USs in a different context may reflect a weakening of the original CS-US association acquired during acquisition (for a similar argument see and Thomas & Ayres, 2004; Thomas, Longo, & Ayres, 2005). The current finding that the presentation of unpaired USs during extinction also slowed re-acquisition is consistent with this interpretation.

In group Standard, the results observed for ratings of CS pleasantness and arousal deviate from those seen for electrodermal responses in that there was no evidence for contextual renewal. This is surprising in that renewal of rated CS pleasantness has been shown in studies of evaluative conditioning (Luck & Lipp, 2020). It may reflect that ratings of CS pleasantness and arousal in context A were assessed after renewal, so after eight extinction trials had been presented in the original acquisition context. Thus, renewal may have been observed in ratings of CS pleasantness and arousal if they were assessed prior to renewal or online and simultaneously with the electrodermal responses (but see Lucas et al., 2018). On the other hand, past research has shown that ratings of CS pleasantness require more trials to extinguish than do measures of outcome expectancies (Lipp & Purkis, 2006; Aust, Haaf, & Stahl, 2019), but this may have been accelerated after the prior extinction training in context B.

Relating back to the accounts offered by Bouton et al. (2004) and Gershman et al. (2013) to

explain the effects of presenting paired or unpaired USs during extinction, the current results do not seem consistent with the explanation offered by Gershman et al. (2013). One might expect that a context change is sufficient to trigger the formation of a new latent state which protects the fear memory created during acquisition from interference. It should be noted, however, that Gershman et al's account was developed to explain the effects of occasional CS-US trials during extinction and may not extend to the effects of additional, explicitly unpaired US presentations. Bouton et al's (2004) trial sequencing account seems better suited to accommodate the findings in group Unpaired, as one may argue that participants learned to expect CS alone trials after presentations of the US. However, this would require the additional assumption that trial sequence learning generalizes across contexts which is not implausible as Bouton et al's (2004) original experiments were conducted across several days which one might argue make for different temporal contexts.

The current results indicate that presenting USs unpaired during extinction can eliminate contextual renewal of electrodermal responding. This is very exciting as it potentially can widen our understanding of the processes that drive extinction. They also have applied promise as the extent to which treatment gains achieved during exposure therapy in the clinic, a context that differs from that of acquisition and potentially from contexts encountered after treatment, are maintained over time is a major concern in clinical practice. However, the clinical utility of presenting occasional USs during exposure therapy seems limited in that re-exposure to the events that may have led to the development of an anxiety disorder is not feasible or ethical. There may however be approaches that will achieve the same aim such as the use of scaled down versions of the US to facilitate extinction training similarly to the manner that USs presented at a lower intensity were suitable retrieval cues to reactivate a fear association (Thompson & Lipp, 2017; Liu et al., 2014) or of high intensity generalization stimuli that resembled the CSs which were as effective as presentations of the CSs during extinction training (Struyf et al., 2018). Alternatively, it may be feasible to combine imagery-based exposure of the US

with exposure to the CS alone in an attempt to approximate the unpaired US presentation procedure employed in the current study (McEvoy et al., 2020). Further studies are required that test whether occasional presentations of stimuli resembling the US but of different intensity or imagery-based procedures are as effective as occasional presentations of the US during extinction.

The current study replicates and extends past findings that presenting the US unpaired during extinction training will reduce return of conditional electrodermal responses, even if extinction is conducted in a context that is different to that of acquisition. Presenting additional USs did not affect differential self-report ratings of CS pleasantness or arousal but prolonged elevated ratings of anxiety which declined after the US was no longer presented. Finding electrodermal renewal after standard extinction, but not after extinction with additional unpaired USs, may suggest that rather than merely strengthening extinction learning, presenting additional USs without the CS may interfere with the CS-US association that was acquired during acquisition, even if these US presentations occurred in a different context. These highly promising findings warrant further elaboration to delineate the mechanism that determines how the presentation of additional USs during extinction prevents the return of fear.

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Tables

Table 1: Demographic and self-report data in the three groups.

	Paired	Unpaired	Standard	
Age (SD)	20.86 (4.77)	19.45 (3.75)	20.61 (4.04)	$F(2,97) = 0.977$, $p = .380$
Gender (F/M)	29/6	24/5	24/12	$\chi^2(2) = 3.39$, $p = .183$
Aware (Y/N)	35/0	29/0	35/1	$\chi^2(2) = 1.80$, $p = .407$
STAI-S	46.17 (10.60)	44.48 (12.85)	45.33 (13.21)	$F(2,97) = 0.151$, $p = .860$
STAI-T	47.40 (12.09)	43.79 (9.15)	42.06 (11.04)	$F(2,97) = 2.19$, $p = .117$
MFQ	8.23 (5.67)	6.69 (4.39)	6.67 (5.19)	$F(2,97) = 1.03$, $p = .360$
PSWQ	57.14 (15.82)	55.34 (14.78)	51.36 (14.15)	$F(2,97) = 1.39$, $p = .254$
MASQ	94.37 (23.92)	88.93 (16.56)	88.89 (25.12)	$F(2,97) = 0.669$, $p = .514$
IUS-12	34.28 (9.75)	30.90 (7.04)	29.94 (7.27)	$F(2,97) = 2.73$, $p = .071$

Aware: Could participants name the animal paired with the US; STAI-S: State-Trait Anxiety Inventory

– state score; STAI-T: State-Trait Anxiety Inventory – trait score; MFQ: Mood and Feelings

Questionnaire total score; PSWQ: Penn State Worry Questionnaire total score; MASQ: Mood and

Anxiety Symptom Questionnaire total score; IUS-12: Intolerance of Uncertainty Scale, short version

Figure Captions

Figure 1: Event sequence in the three experimental groups. Ratings of CS pleasantness and arousal, and of subjective anxiety were assessed before and after habituation and after Acquisition, Extinction, Renewal, and Re-acquisition. Ratings of US intensity and pleasantness were assessed after acquisition and extinction.

Figure 2: Electrodermal first interval responses as a function of group, conditional stimulus, and experimental phase (error bars represent standard errors of the mean).

Figure 3: Electrodermal last interval responses as a function of group, conditional stimulus, and experimental phase (error bars represent standard errors of the mean).

Figure 4: Ratings of subjective anxiety assessed before and after Habituation and after Acquisition, Extinction, Renewal, and Re-acquisition as a function of group (error bars represent standard errors of the mean; * indicates significant difference between groups).

Figure 5: Ratings of CS⁺ and CS⁻ pleasantness assessed before and after Habituation and after Acquisition, Extinction, Renewal, and Re-acquisition as a function of group (error bars represent standard errors of the mean; * indicates significant difference in rated CS pleasantness between CS⁺ and CS⁻).

Figure 6: Ratings of CS⁺ and CS⁻ arousal assessed before and after Habituation and after Acquisition, Extinction, Renewal, and Re-acquisition as a function of group (error bars represent standard errors of the mean; * indicates significant difference in rated CS arousal between CS⁺ and CS⁻).

Figure 1

	Habituation (Context A)	Acquisition (Context A)	Extinction (Context B)	Break (10 min)	Renewal (Context A)	Re-acquisition (Context A)
Standard			24CS ⁺ /24CS ⁻	Remove electrode		
Paired	4 CS ⁺ /4 CS ⁻	8 CS ⁺ -US/8 CS ⁻	19CS ⁺ /5CS ⁺ -US/24CS ⁻	leads/ complete	4CS ⁺ /4CS ⁻	4CS ⁺ /4CS ⁺ -US/8CS ⁻
Unpaired			24CS ⁺ /5US/24CS ⁻	puzzle		

Figure 2

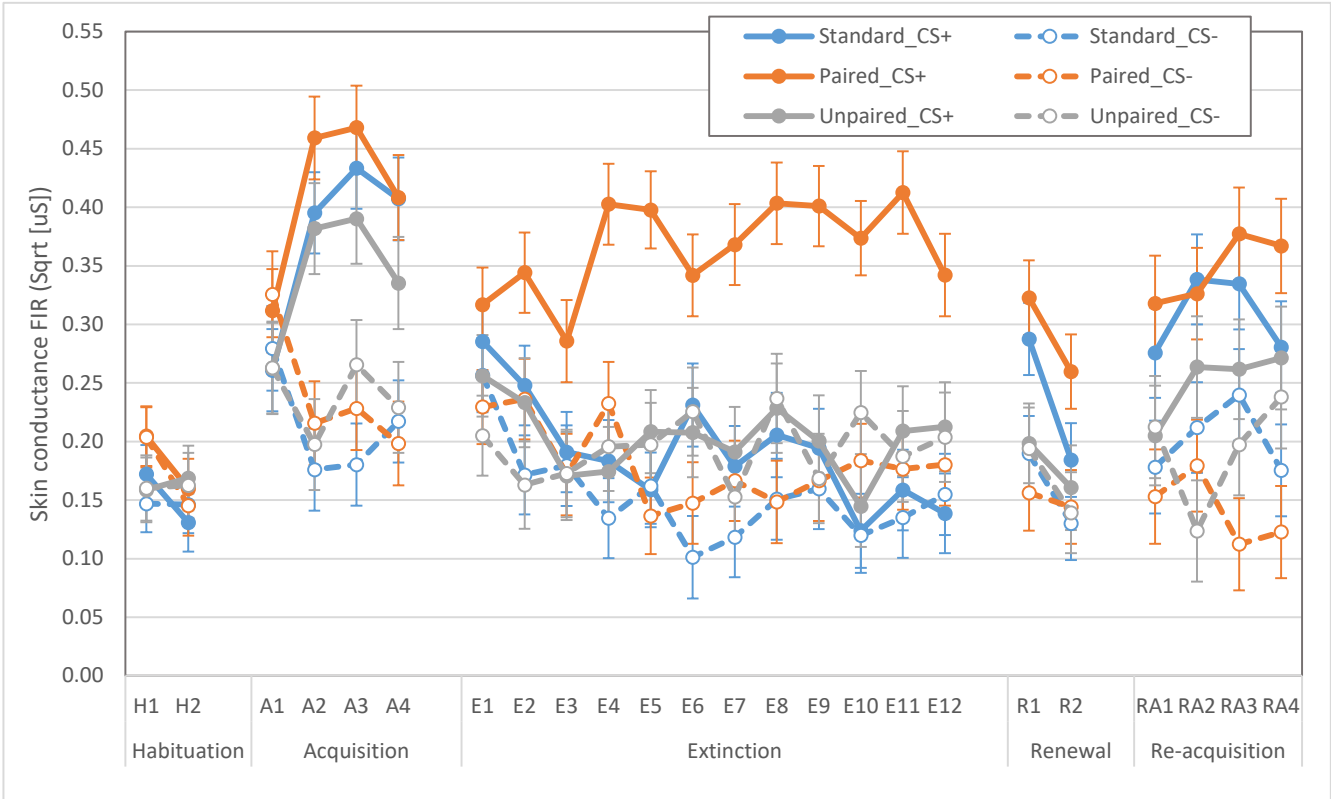


Figure 3

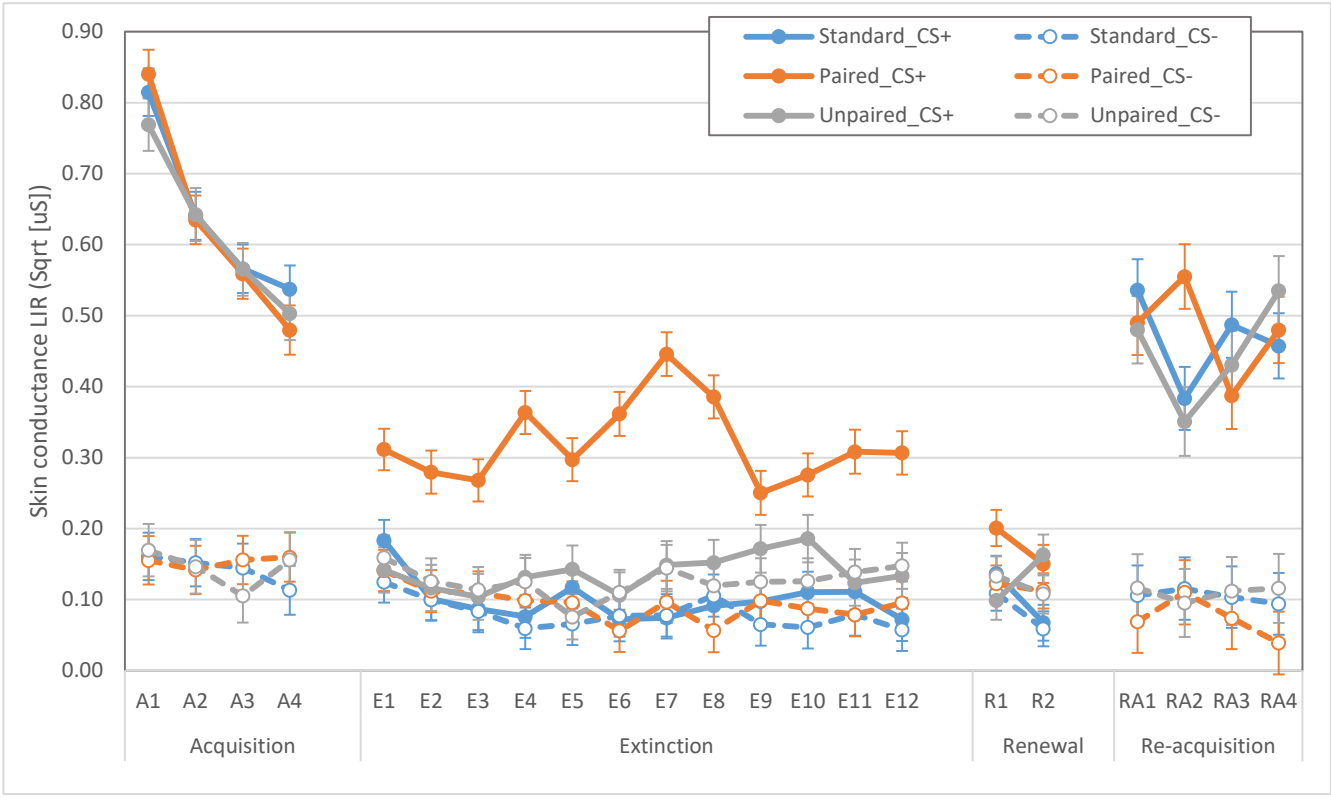


Figure 4

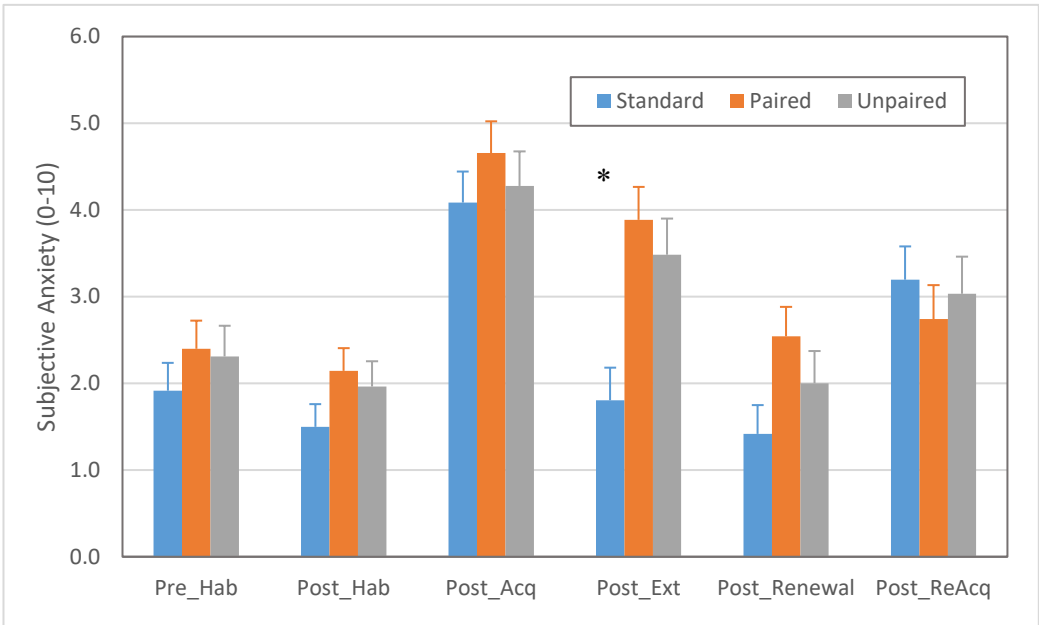


Figure 5

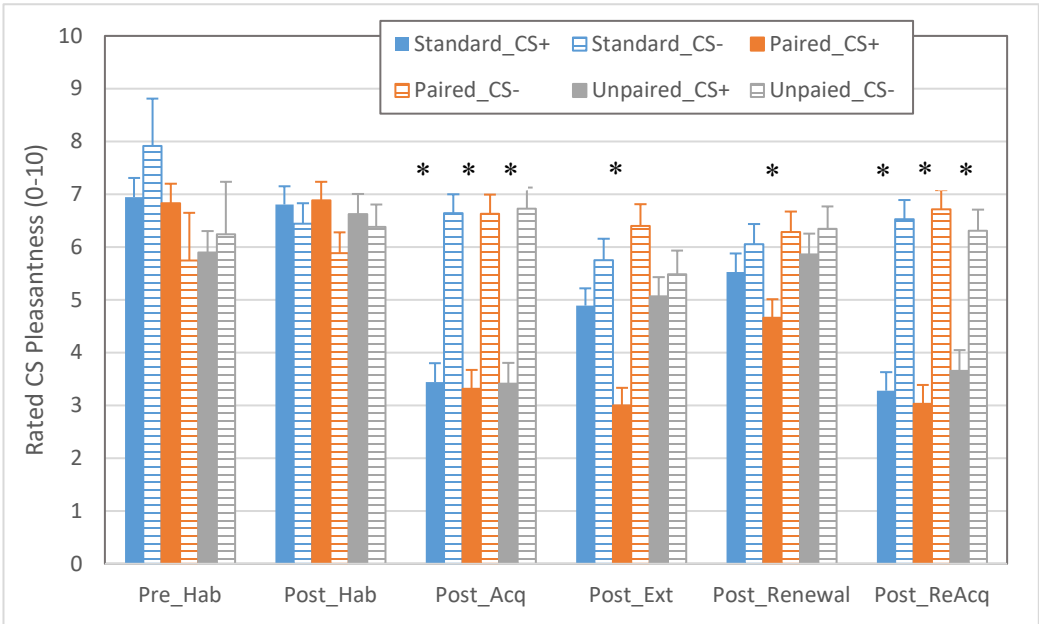


Figure 6

